

Reproductive Behavior in the Squid *Sepioteuthis australis* From South Australia: Interactions on the Spawning Grounds

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Abstract. Squid behavior is synonymous with distinctive body patterns, postures, and movements that constitute a complex visual communication system. These communications are particularly obvious during reproduction. They are important for sexual selection and have been identified as a potential means of species differentiation. Here we present a detailed account of copulation, mating, and egg deposition behaviors from *in situ* observations of the squid *Sepioteuthis australis* from South Australia. We identified four mating types from 85 separate mating attempts: “Male-upturned mating” (64% of mating attempts); “Sneaker mating” (33%); “Male-parallel” (2%); and “Head-to-head” (1%). Intervals between successive egg deposition behaviors were clearly bimodal, with modes at 2.5 s and 70.0 s. Ninety-three percent of egg capsules contained 3 or 4 eggs (mean = 3.54), and each egg cluster contained between 218 and 1922 egg capsules (mean = 893.9). The reproductive behavior of *S. australis* from South Australia was different from that described for other cephalopod species. More importantly, comparison between these results and those for other populations of *S. australis* suggests that behavior may differ from one population to another.

Introduction

Mate choice arises from behavioral interactions that generate selection for gender-specific traits (secondary sexual characteristics) (Ryan, 1997). Differences in reproductive success of individuals are, in turn, typically held to be caused by competition for mates (Andersson, 1994; Ryan,

1997). In systems where female choice is prevalent, sexual selection should favor conspicuous male traits that allow males to out-compete (directly or indirectly) other males (Andersson, 1994). These traits can be morphological, physical, or behavioral (Parker, 1984; Andersson, 1994; Birkhead and Parker, 1997; Ryan 1997).

In cephalopods, secondary sexual characteristics primarily consist of differences in body size, body patterns, sucker size, gonad shape or color, and sometimes photophores (Hanlon and Messenger, 1996). In squid, behavior comprises rapid body pattern changes that result from alterations in chromatic, postural, or locomotor components of behavior (Mather and Mather, 1994; Hanlon and Messenger, 1996). These behavioral patterns form a complex visual communication system. Interpreting this communication system is fundamental to understanding the processes of sexual selection in these species.

Analysis of reproductive behavior can be important when discriminating closely related species (Hanlon, 1988). Although camouflage patterns are likely to be highly conserved due to responses to common predators, reproductive communication is likely to have species-specific signals. Roper and Voss (1983) documented the range of morphological characters for species descriptions of cephalopods, and Hanlon (1988) proposed additional behavioral characters for identification. Some of the characters that Hanlon (1988) cites as being important are intraspecific agonistic behavior, mating behavior, spawning and egg care behavior, and chromatic components of body patterns. In line with these criteria, cephalopod taxa such as the squid *Sepioteuthis lessoniana* from Japan are now being reviewed and reclassified (Segawa *et al.*, 1993a).

Although still regarded as a single species, geographically different populations of *S. lessoniana* are thought to be taxonomically different (Segawa *et al.*, 1993a, b; Izuka *et*

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al., 1994, 1996a, b). Differences between these populations occur both at a genetic level (Izuka *et al.*, 1994) and at a population level in differences between reproductive behavioral characteristics such as egg deposition (Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994).

Similar uncertainty about genetic differences between geographically isolated populations of *S. australis* has arisen recently. Allozyme analysis of Australian and New Zealand populations of this species indicates that they are genetically distinct (Triantafillos and Adams, 2001). However, owing to the lack of comparative data on the behavior of this species in New Zealand and Australia, it is not known whether the genetic differences are expressed as behavioral differences.

The aim of this study was to identify and describe the reproductive behavior of *Sepioteuthis australis* from South Australia. We recently cataloged (as an ethogram) the suite of reproductive body pattern components for this species from South Australia. (Jantzen and Havenhand, 2003a). Here, we report results of underwater digital video imaging, photographs, and field notes that document the reproductive behavior of *S. australis* on spawning grounds over three consecutive spawning seasons. Our descriptions include previously unreported reproductive behaviors. These results are compared with previous descriptions for this, and other, squid species to identify aspects of reproductive behavior that might provide insights into secondary sexual selection in squids and the evolutionary significance of these reproductive strategies.

Materials and Methods

Mating behavior was observed during daylight hours on spawning grounds between Marino and Hallett Cove, South Australia (138° 29' E, 38° 02' S) between December 1999 and March 2002. All data were collected during the main spawning season each year (September to March). The substrate on the spawning grounds consists of patches of bare sand and rock interspersed with seagrass (*Amphibolis antarctica*) and brown macroalgae (*Sargassum* spp.) Reproductive activity of squid on spawning grounds was identified by visual observation from the surface at known locations, and by the activity of recreational and professional fishermen. Reproductive activity was observed by scuba diving and directly from the surface in less than 4.5 m of water. The presence of divers close (< 30 cm) to reproductively active individuals caused no apparent alteration in squid behavior (when compared with behavior of individuals observed from afar). Observations were therefore routinely made at a distance of less than 2 m. Still photographs of mating and spawning behavior were taken with a Nikon V camera (Nikon Corporation, Melville, NY), and video images were recorded with a Sony TV120 digital video camera (Sony Corporation, New York) in an Amphibico

housing (Amphibico, Quebec, Canada). Video sampling followed the protocol of Martin and Bateson (1993) for focal-animal sampling, with additional *ad libitum* video sampling of specific behaviors. Detailed notes of reproductive activity were recorded for every observation period and compared with video and still images of behavior for the same period.

We previously identified the reproductive body patterns of *Sepioteuthis australis* (Jantzen and Havenhand, 2003a). The nomenclature of these patterns follows the convention of capitalizing the first letter of formally defined patterns of behaviors (Hanlon and Wolterding, 1989). Terminology applied to the physical characteristics of squid follows that described by Hanlon and Messenger (1996, fig. 2.1, p. 13). Frame-by-frame sequences of selected behaviors were obtained and analyzed using Final Cut Pro software (Apple Computer, Cupertino, CA) at a frame rate of 25 frames per second.

Durations between the completion of "Egg passing" and egg deposition, between egg deposition and "Peristaltic arm flare," and between successive observations of "Peristaltic arm flare" were analyzed using a one-way ANOVA to investigate differences between females. To meet the assumption of homogeneity of variance, data for durations between "Egg passing" and egg deposition were square-root transformed prior to analysis. These analyses were conducted to determine if these behaviors were consistent between females.

Results

Over a period of three consecutive spawning seasons, we observed more than 550 reproductively active individuals of *Sepioteuthis australis* in over 75 hours underwater. Observation sessions lasted as long as 120 min at a time, and the number of reproductively active squid present at any one time ranged from 2 (a single spawning pair) to more than 45 per dive. The length of time that each sex remained on the spawning grounds could not be quantified, because the size of the spawning grounds exceeded the visible range underwater. However, on all but two occasions, focal females remained in a localized area on the spawning ground throughout the observation period. In the two other instances, females swam out of view while being observed, and we do not know whether they remained on the spawning grounds. Furthermore, all observations were conducted during daylight, so we do not know if reproductive activity continued at night. Direct counts of sex ratio on each dive (and subsequent checking of these counts from the video images) showed a male-biased sex ratio between 1:1 (a single pair) and 3:1 (>4 individuals). Females were typically paired with a male, while several unpaired males swam amongst the paired individuals.

Mating

Four mating types were observed during 85 mating attempts. Mating types were classified as paired "Male-up-turned mating" (PU), paired "Male-parallel mating" (PM), paired "Head-to-head mating" (PH), and "Sneaker mating" (SM). Paired mating types occurred only between paired individuals, whereas "Sneaker mating" comprised all attempts by unpaired males to mate with paired females. Of the four mating types, PU and SM were most frequently observed (64% and 33%, respectively, Fig. 1), with PM and PH seen on only 2% and 1% of matings respectively (Fig. 1).

Paired Male-up-turned mating (PU). Paired Male-up-turned mating occurred most frequently (54/85 matings, Fig. 1), with a mean inter-mating interval of 7.09 min (SEM = 3.27 min, $n = 11$). In all cases, males swam into a position over the female prior to PU while showing "Mantle margin stripe," "Dark arm stripes," "Fin stripe," "Shaded eye," and "Rigid arms" body pattern components (Figs. 2A, 3). On six occasions, a male was seen to show up to five rapid "Lateral splayed arms" components in quick succession while above the female. This "Lateral splayed arms" behavior did not appear to evoke a response by the female.

Once above the female, males rotated 180° around the longitudinal axis (Fig. 2B). Simultaneous with this rotation,

the hectocotylized arm (left 4th arm) began moving back toward the funnel, and the right 4th arm moved toward the buccal region of the female (Fig. 2B). Once the animal was completely upside-down, spermatophores were ejected through the funnel (Fig. 2C) and gathered with a sweeping action of the hectocotylized arm across the funnel. This arm was then extended beside the right 4th arm (positioned with the tip in the female buccal region; Fig. 2D), and spermatophores were delivered into the buccal region of the female (Fig. 2E). The male then rotated back to the normal swimming position (Fig. 2F). From initial rotation of the male to completion of mating took less than 3 s.

Throughout PU, females showed "White dorsal stripe," "Golden epaulettes," and "Rigid arms" body pattern components (Figs. 2A, 3). Occasionally the posterior mantle of the female was seen to move downward 30°–40° from horizontal as the male began to rotate around to the upside-down position. PU did not occur before every deposition of an egg capsule (Fig. 3); however, this component was always observed after "Egg passing" (see below) and before egg deposition. Throughout PU, females were usually within 1 m of an egg cluster and within one body length of the substrate.

Boal and Gonzalez (1998) describe four classes of PU mating for *S. lessoniana*: "Pre-mating behavior" (mutual swimming of spawning pairs in a back-and-forth motion),

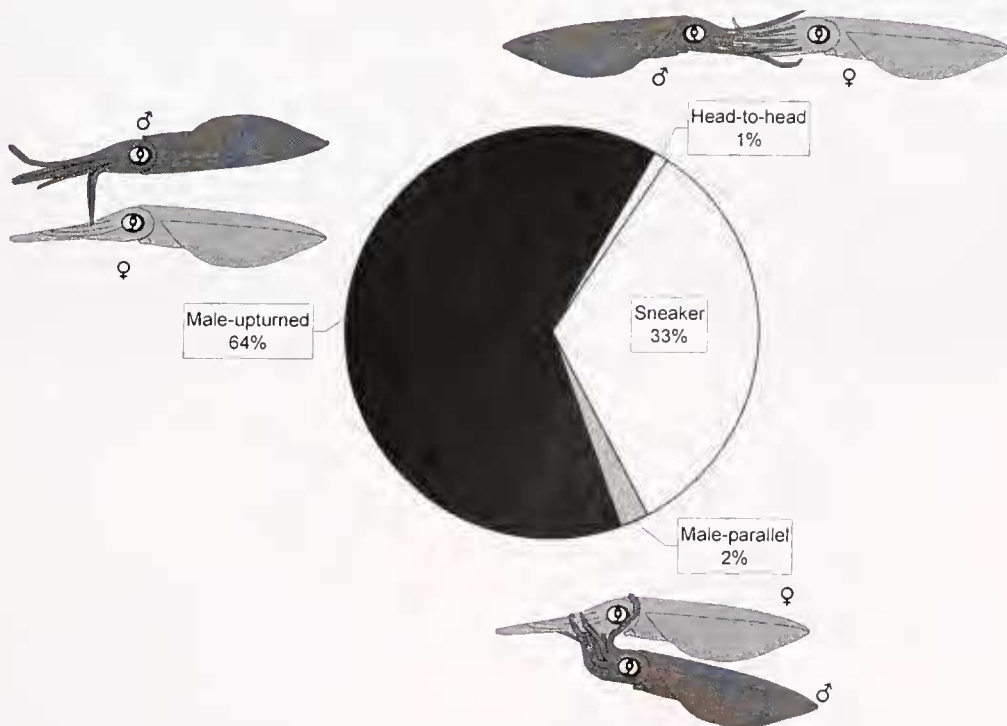


Figure 1. Percentage (of all matings) of each of the four mating types identified in *Sepioteuthis australis*, with illustrations of the three mating types between paired individuals.

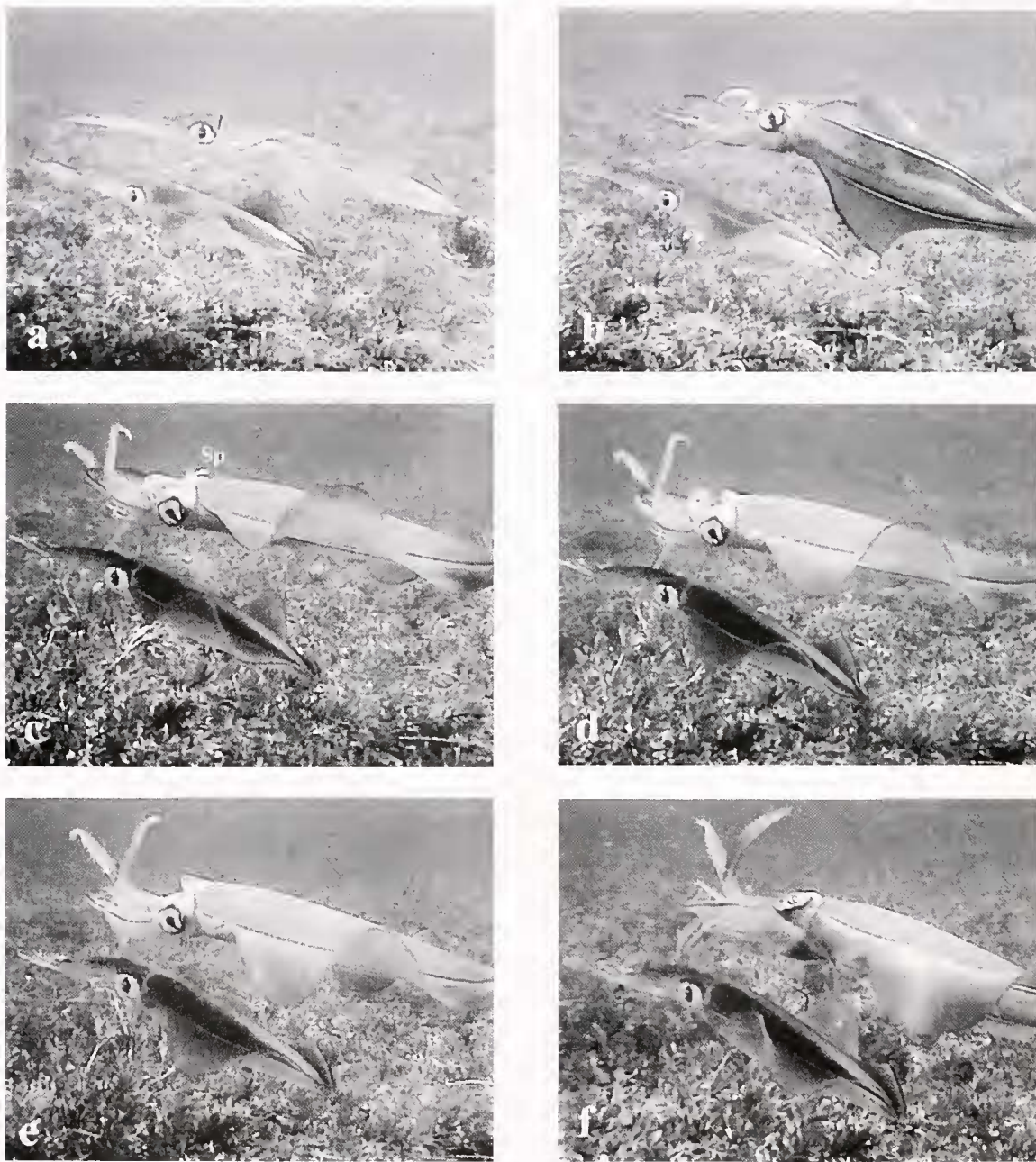


Figure 2. Six-frame sequence of "Male-upturned mating" behavior in *Sepioteuthis australis*. The male (top) swims into a position over the female (bottom): a). The male then rotates to the upside-down position (b) and gathers spermatophores (Sp) from the funnel with the left 4th (hectocotylized) arm (c). The hectocotylized arm then moves down the right 4th arm that is positioned in the buccal area of the female (d) and deposits spermatophores in this area (e). Copulation is complete, and the male rotates back to the normal swimming position (f). Total time elapsed = 3 s.

"Flip" (whereby the male rotated around into an upside-down position), "Contact" (when the male physically contacted the female), and "Attempt" (when no contact was made with the female by the male while in the upside-down position). Only "Pre-mating behavior," "Flip," and "Contact" classes of PU had been previously described in *S. australis* (Jantzen and Havenhand, 2003b). We also ob-

served the "Attempt" class of PU mating, in which mating was clearly unsuccessful (*i.e.*, no physical contact was made with the female).

A few spermatophores (about 3–5) were seen being transferred to the female during PU. Direct counts of the number of spermatophores transferred were impossible due to the speed of PU mating (<3 s). Consequently, these numbers

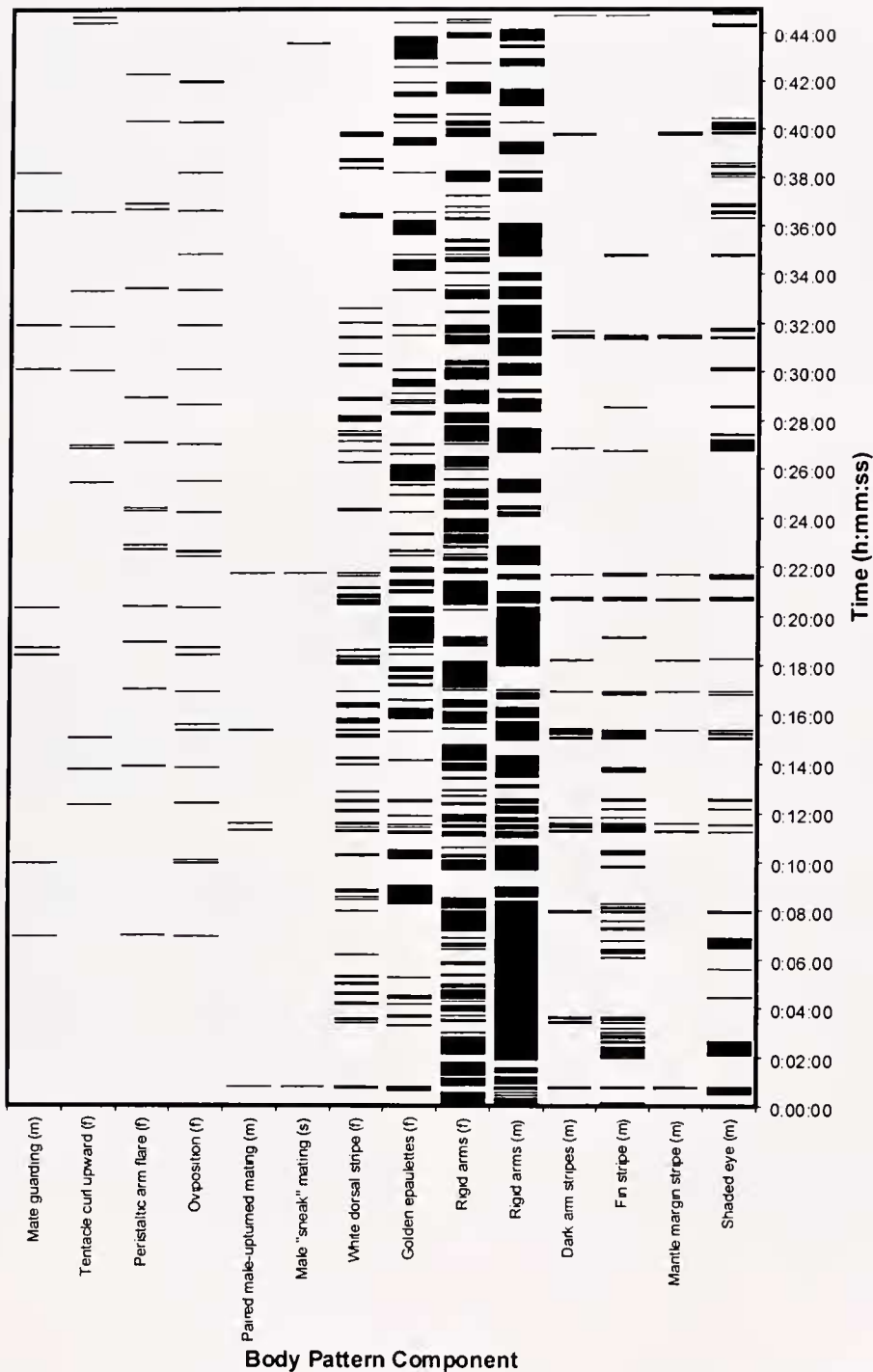


Figure 3. Signal periodicity and duration of selected components associated with egg deposition and mating from a single spawning pair of *Sepioteuthis australis*. Activity was recorded for a continuous focal sampling period of 45 min. Each horizontal bar represents the activity of a single body component from a single animal at any given time. Letters in parentheses indicate sex of the focal animal from which each body pattern component is recorded (m = paired male, f = paired female, s = sneaker male).

were estimated from analysis of digital images of two successful mating attempts and two unsuccessful mating attempts. In the two successful mating attempts, three and

five spermatophores were counted. The unsuccessful mating attempts resulted in spermatophores being released into the water column, where they could be counted readily. In these

unsuccessful attempts. spermatophoric reaction (the process by which sperm are ejected from the spermatophore; Mann *et al.*, 1966) had occurred. Both times, three coagulated strands of sperm were identified.

Paired Male-parallel mating (PM). Two PM attempts were observed (2.4% of all mating attempts). On both occasions, both sexes showed the "Plain" chromatic component prior to, and throughout, PM. Our observations of PM were very similar to reports of Male-parallel mating in other squid species (*e.g.*, Drew, 1911; McGowan, 1954; Arnold, 1962). The transfer of spermatophores to the female was not seen, and therefore we could not ascertain whether (or where) spermatophores were deposited on or in the female.

Paired Head-to-head mating (PH). This behavior was seen on only one occasion. The male swam rapidly toward the female head-on and grasped her arms and tentacles. The male remained in this position for less than 1 s before the pair separated. "Plain" was the only chromatic component seen in both sexes, and the transfer of spermatophores was not seen.

Sneaker mating (SM). On 28 occasions, an unpaired male attempted to mate with a paired female. These events were classed as SM, and occurred mostly while a paired female was attempting to deposit an egg capsule at an egg cluster, or simultaneously with the mating attempt of a paired male (Fig. 3). Four types of SM were observed: "Sneaker males" darted amongst the vegetative substrate and made contact with a female while she was at an egg cluster. Sneaker males mated in an upside-down position (consistent with the behavior of the paired male in PU mating, described above) at the egg cluster, but in the "Male-parallel" position if the paired male was "Parrying" a second unpaired male. (Note that in all cases, "Plain" was the only chromatic body pattern consistently shown by the sneaker males in these three SM types.) The fourth SM type involved sneaker males appearing to mimic a paired female. This was seen twice, and no agonistic response was shown by the paired male as the sneaker male approached the paired female. Following these mating attempts, a second unpaired male was seen attempting to mate with the sneaker males. The prominent chromatic body patterns shown by the sneaker males were "Dark mantle," followed by "Dorsal white stripe" as well as "Golden epaulettes" and "Rigid arms." These latter three body pattern components are typical of paired females throughout PU (see above).

General mating behavior. Spermatophores were generally found deposited in the buccal cavity of females; however, several females were observed with spermatophore capsules affixed to the head, arms, or dorsal mantle. The copulation attempts that led to the placement of these spermatophores were not seen; however, in one instance, a sneaker male attempted to make contact with a female on the head. The placement of spermatophores was not identi-

fied in this instance. Given that in all paired matings (and paired mating attempts), spermatophores were never seen to be placed outside the buccal cavity, it seems likely that these extra-buccal spermatophores were the result of Sneaker mating.

It was not uncommon for PU and SM to occur simultaneously (Fig. 3); on most of these occasions, females rapidly jetted away from the simultaneous mating attempts.

Egg deposition

When a female approached an egg cluster, her tentacles folded back laterally (Fig. 4a-e) as she descended toward the substrate and deposited a new egg capsule with her arms. In all cases ($n = 226$), attachment of egg capsules was completed in less than 2 s. Paired males regularly remained a few centimeters above the female as she attached egg capsules to a cluster ("Mate guarding"). New egg capsules appeared translucent immediately upon deposition (Fig. 4f), and the number of eggs within each newly deposited capsule was clearly visible. No more than three females were seen contributing egg capsules to each cluster. The chromatic components of females depositing egg capsules in a cluster were rarely seen, because the female was routinely obscured from view by the substrate.

The interval between deposition of successive egg capsules showed a bimodal distribution (Fig. 5). Modes in interval frequency occurred at 2.5 s and 70.0 s. This pattern was evident for each female recorded ($n = 11$ females). Short durations always occurred singly (*i.e.*, were followed by at least one long duration; Fig. 3).

Egg capsules contained 5 eggs or less with 93% of egg capsules containing 3 or 4 eggs (Fig. 6). The average number of eggs per capsule was 3.54 (SEM = 0.040, $n = 300$ capsules). The average number of capsules per egg cluster was 893.9 (min = 218, max = 1922, $n = 9$).

Following egg deposition, females were often seen radially flaring their arms and tentacles from the base to the tips while simultaneously pulsing a jet of water across the arms and tentacles ("Peristaltic arm flare"; Jantzen and Havenhand, 2003a). Often, small white particles were seen rapidly expelled from the arms of the females as a result of this behavior. This "Peristaltic arm flare" occurred multiple times (commonly twice and occasionally as many as 4 times) within females. The first such event typically occurred within 20 s after the completion of egg deposition (84% of observations, Fig. 7). No significant differences in the duration of the interval between the completion of egg deposition and the first observed "Peristaltic arm flare" were found among separate females (one-way ANOVA, $F_{9,247} = 1.630$, $P > 0.05$). This supports our observations that duration of the interval between completion of egg deposition and the first observed "Peristaltic arm flare" was consistent between females. Successive "Peristaltic arm flare" compo-



Figure 4. Sequence of "Egg deposition" behavior in *Sepioteuthis australis*. (a) After moving into a position over the egg cluster, the female moves forward towards the egg cluster and curls both tentacles back dorsally, with the suckers on the club facing out (b). Upon nearing the cluster, both tentacles then fold back laterally (c) so that the club of each tentacle is adjacent to each eye (d). The female then attaches a new egg capsule to the existing egg cluster (e). Total time elapsed = 3 s. Following deposition, egg capsules are translucent for a short time, and individual eggs (~ 10 mm long) are clearly visible until the capsule becomes opaque (f).

nents were rapid (27% within 2 s and 58% within 10 s, Fig. 8). Again, no significant difference in the duration of this interval was found among 10 females (one-way ANOVA, $F_{9,91} = 0.496$, $P > 0.05$), suggesting that the interval between successive "Peristaltic arm flare" components is similar between females.

Egg passing

"Egg passing" denotes the process by which female squid pass eggs and associated egg capsule material from within the mantle cavity into the arms in readiness for deposition. The beginning of "Egg passing" was defined when the

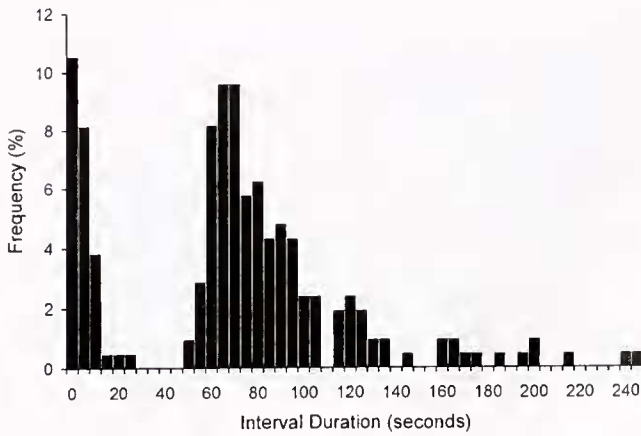


Figure 5. Frequency (%) of interval between the deposition of successive egg capsules in female *Sepioteuthis australis* ($n = 209$ durations).

funnel was directed upward toward the center of the ventral arm bases. A series of peristaltic movements passed through the funnel from the mantle toward the arms for, on average, 25.8 s ($n = 22$, SEM = 0.6 s). Throughout "Egg passing," the ventral arm bases extended to about double their normal size (Fig. 9). Within 5 s of the completion of "Egg passing," the ventral arm bases returned to the size observed prior to "Egg passing" behavior. Neither eggs nor egg capsule material was seen throughout "Egg passing," but following this behavior the arms of the female remained in a "Rigid arms" position until the egg capsule was deposited in the cluster.

The mean interval between the completion of "Egg passing" (the moment when the funnel resumed the "normal" position) and the beginning of egg deposition was 30.2 s (SEM = 4.5 $n = 30$ observations from 8 females). This duration did not differ significantly between females (one-way ANOVA, $F_{7,22} = 1.876$, $P > 0.05$), which agreed with our observations that this behavioral interval was consistent between females.

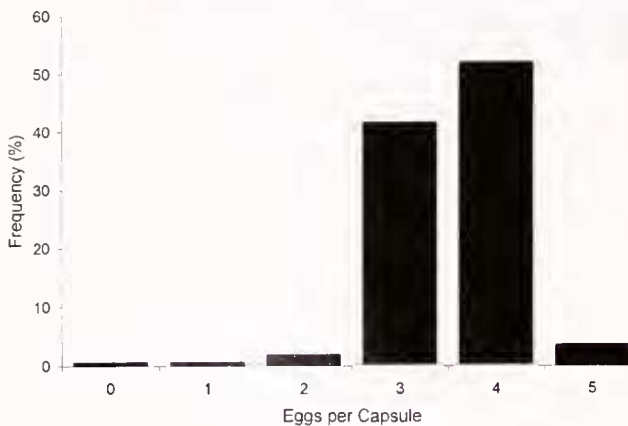


Figure 6. Frequency distribution (as a percentage) of egg number per capsule for *Sepioteuthis australis* ($n = 300$ capsules).

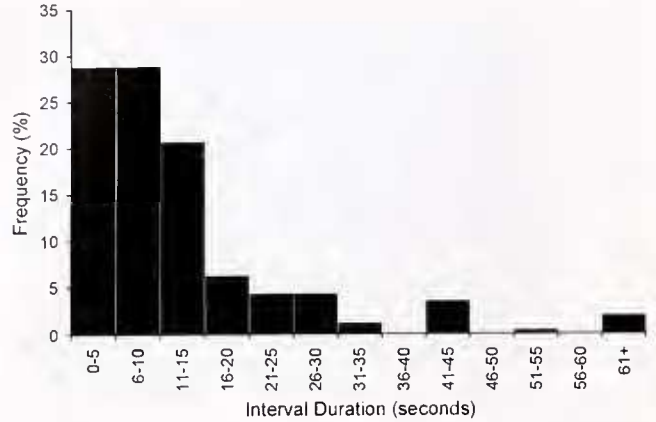


Figure 7. Frequency of observations of interval duration between conclusion of egg deposition and "Peristaltic arm flare" behavior in females of *Sepioteuthis australis* ($n = 257$ intervals).

Male agonistic contests

The behavior of males throughout agonistic contests was consistent with the observations of Jantzen and Havenhand (2003b). Paired males spend considerable time positioning themselves between unpaired males (these attempting to displace paired males from their mate) and the paired female ("Parrying"). "Parrying" is considered to be the very early stages of agonistic contests between rival males and was not associated with any specific chromatic body pattern components. As these contests intensified, rival males began "Fin beating" (described as "swimming fight" in Jantzen and Havenhand, 2003b). "Fin beating" occurred when both males extended their arms and tentacles and collided while swimming backwards. At this time both males showed "Dark mantle" and "Iridescent sclera" chromatic body pattern components. "Fin beating" was quite forceful between males, and the collision of mantles and fins was intense. In

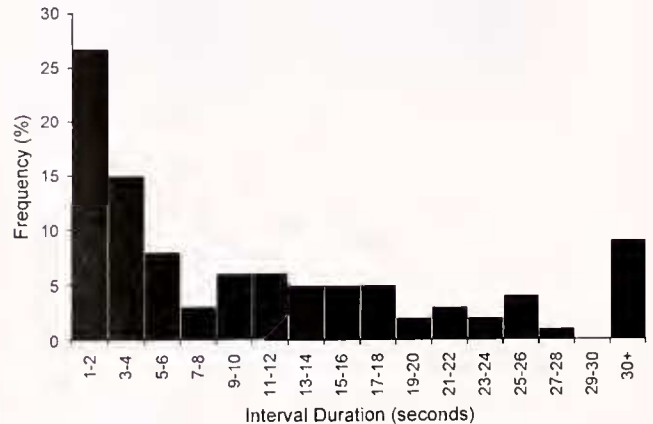


Figure 8. Frequency of observations of interval duration between successive "Peristaltic arm flare" behaviors occurring between successive egg depositions in females of *Sepioteuthis australis* ($n = 101$ intervals).

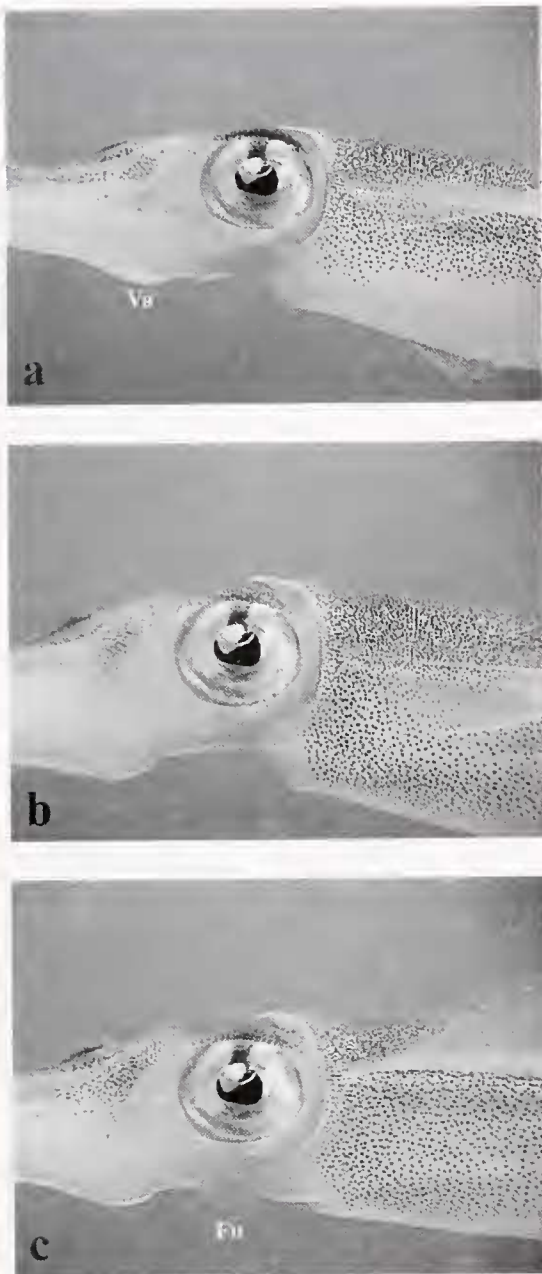


Figure 9. Three-frame sequence of "Egg passing" behavior in females of *Sepioteuthis australis*. Throughout "Egg passing," the ventral arm region (Va) becomes extended, and the funnel (Fu) is positioned flat against the underside of the head (immediately below the eyes; a). "Egg passing" is completed when the ventral arm region becomes substantially engorged (b), and the funnel moves back to the "normal" swimming position (c). Total time elapsed = 3 s.

all fights observed ($n = 67$), the paired male was "victorious" such that it remained paired with the female at the completion of the fight. The unpaired was always the "loser," eventually swimming away from the pair.

Paired males also "Charged" rival males that had approached the paired female. In all observations of "Charg-

ing" ($n = 7$), the paired male swam rapidly at the unpaired male, striking it with the tentacles while radially flaring all arms. This is an intense agonistic encounter by a paired male and appeared similar to that described for *L. pealeii* (King *et al.*, 1999) and *Loligo forbesi* (Porteiro *et al.*, 1990). Unpaired males always retreated following this contact.

Discussion

Mating behavior and sexual selection

About 67% of all observed mating attempts were made by paired males (Fig. 1). This indicates that paired males are able to out compete unpaired sneaker males for access to females and that paired mating has probably evolved as a more successful male mating tactic. Within paired males, three mating types were observed ("Male-upturned mating," "Head-to-head mating," and "Male-parallel mating"), with some males mating with a female in all three paired mating positions. Previously, no more than two mating positions had been reported by paired males within a single squid species (table 6.2 in Hanlon and Messenger, 1996). By far the most common paired mating type was "Male-upturned mating" (95.5% of paired mating attempts; 64% of total mating attempts), with "Head-to-head mating" (1.5% of paired, 1% of total), and "Male-parallel mating" (3% of paired, 2% of total) constituting considerably fewer mating attempts. This behavior contrasts with that of other species of squid such as *Loligo plei*, in which "Head-to-head mating" occurs before adults reach the spawning ground and "Male-parallel mating" occurs only when individuals have arrived at the spawning ground (Hanlon and Messenger, 1996). In *Sepioteuthis australis*, all three mating types were seen on the spawning ground (no data are available for possible mating behaviors prior to reaching the spawning grounds). This indicates that individual paired males of this species show considerable flexibility in mating positions. Importantly, there also appears to be flexibility in the placement of spermatophores by males. Spermatophores were most commonly deposited in the female buccal region, but they were occasionally found in the female's mantle or on her head, arms, or dorsal mantle; they may even have been placed directly onto egg capsules as they were deposited.

In addition to outcompeting smaller males, paired males may also increase their copulation frequency as a result of mating flexibility. Different mating strategies are possibly a response that allows an individual to select the most appropriate mating strategy for the surrounding environment (Patridge and Halliday, 1984). The environmental variables influencing the mating frequency of paired male squid are likely to include not only female receptivity but also sex ratio on the spawning ground, density of reproductively active individuals, number of "sneaker" males, and susceptibility to predation while mating (although only the latter of these factors has been quantified; Smale *et al.*, 2001). It is

reasonable to assume that mating positions have evolved to provide maximum chance of successful copulation while minimizing risk to the mating pair. We have no data relating to predation risk during copulation or the potential selective benefits of different mating types; however, it is noteworthy that "Male-upturned mating" was prevalent at low and high densities (2–45+ individuals in the spawning aggregation), as well as in the presence and absence of sneaker matings.

Thirty-three percent of mating attempts were by sneaker males. Like paired males, sneaker males also showed a degree of mating flexibility in that they attempted to mate in different locations (mostly at the egg cluster but occasionally away from it), in different mating positions ("Male-upturned" and "Male-parallel"), and showing different body patterns (*i.e.*, possible female mimicry). Sneaking behavior of unpaired males is widespread among cephalopods (Hanlon *et al.*, 1994, 1997, 1999b; Hanlon, 1996; Hall and Hanlon, 2002; Jantzen and Havenhand, 2003b); however, despite camouflage and mimicry behaviors being used by cephalopods (Hanlon and Messenger, 1996; Hanlon *et al.*, 1999a; Norman *et al.*, 1999, 2001), female mimicry by sneaker males has not been reported in squid and is known only in the giant cuttlefish *Sepia apama* (Norman *et al.*, 1999). Instances when sneaker males were observed to possibly mimic female behavior involved the chromatic signals "Dark mantle" followed by "Dorsal white stripe," "Golden epaulettes," and "Rigid arms" (these latter three components are characteristic signals of females copulating with paired males). The success of this apparent mimicry was evident from the observations ($n = 2$) that other males attempted to mate these males, and spermatophores were clearly seen being delivered to the recipient male. Given the low success rate of unpaired males in agonistic encounters with paired males (see below), it seems likely that this possible mimicry behavior has evolved so smaller males can approach paired females without instigating potentially harmful or costly agonistic contests with competing males.

It is central to sexual selection theory that differences in the reproductive success of individuals are caused by competition for mates (Andersson, 1982, 1994; Ryan, 1997). It must be remembered, however, that mating success in *S. australis* merely places spermatophores within the buccal region of the female—it does not necessarily result in successful fertilization. Females may mate with many males, and spermatophore longevity can be considerable (>2 weeks; Jantzen and Havenhand, unpubl. data). Consequently, female choice may play a vital role in dictating the fertilization success of sperm from different males.

The only behavior akin to direct female choice of spermatophores observed here was that in which females rejected a mating attempt by rapidly retreating ("Jetting" away). This behavior was seen only in simultaneous mating attempts of paired and sneaker males and not in (undisturbed) paired mating attempts. Consequently it seems

likely that "Jetting" away was a female response to an attempted "Sneaker mating" (SM) by an unpaired male, rather than the specific rejection of a paired mating attempt. This female response to SM suggests that females are actively selecting paired males as preferred mates, which is a form of intrasexual selection (Wiley and Poston, 1996). This will add to the intense competition between males to form pair bonds with females. In systems where female choice is prevalent, sexual selection favors (among other factors) conspicuous behavioral male traits that allow males to out-compete other males (Andersson, 1982, 1994; Parker, 1984; Birkhead and Parker, 1997; Ryan 1997). It is therefore unsurprising to find intense agonistic contests between males to form pairs with females. Unlike female choice, these behavioral competitions between males for mates are a form of intersexual selection (Wiley and Poston, 1996).

In the squid *S. sepioidea*, a female actively accepts or rejects spermatophores placed on her arms or head (Moynihan and Rodaniche, 1982). Although we also saw spermatophore capsules on the head, arm, and dorsal mantle of females, all spermatophores seen transferred during paired matings were deposited into the buccal region. Therefore, it is likely that those spermatophores placed on the head, arms, and dorsal mantle of females resulted from mating attempts by sneaker males.

The relationship between spermatophore placement and fertilization success is very poorly understood for cephalopods in general. In *S. australis*, most SM attempts occurred while females deposited egg capsules in clusters, and any spermatophores deposited were placed on the female or directly onto the egg capsules. Paired mating attempts usually occurred prior to this egg deposition, and spermatophores were deposited primarily in the female buccal region. In squid, sperm must penetrate several millimeters of egg capsule matrix to fertilize eggs, and fertilization is thought to take place only after the egg capsule has been deposited (Arnold, 1971). Therefore, it is expected that the egg capsules from females that had been mated by two different males would routinely contain sperm from both matings, and these sperm would compete to fertilize the eggs (*sensu* Parker, 1970; Birkhead and Parker, 1997). Because sperm from paired matings contact egg capsules earlier (*i.e.*, when in the arms of the female) than those from sneaker matings (*i.e.*, during egg deposition), they have a longer exposure time to the egg capsule. Sperm contact time with eggs has been widely shown to increase fertilization success in sea urchins (*e.g.*, Vogel *et al.*, 1982; Levitan *et al.*, 1991); however, comparable effects have not been tested in cephalopods. It seems likely that, as a result of longer sperm egg contact times, fertilization success of paired matings will be higher than that of sneaker matings, but the extent to which sneaker mating results in successful fertilization in *S. australis* has not been established.

Analytical protocols to detect the paternity of *S. australis*

embryos have recently been developed, and preliminary analysis indicates that egg clusters contain eggs sired by more than one male (L. van Camp, Flinders University; unpubl. data). Similar analyses have yet to be conducted on individual egg capsules. Multiple paternity of eggs within capsules is common in the squid *Loligo pealeii* and *Loligo forbesi* (Shaw and Boyle, 1997; Buresch *et al.*, 2001), but details of the provenance of the males that sired the eggs (sneaker or paired) were not known. Comparable paternity investigation coupled with behavioral analysis similar to that conducted here should provide valuable information about the relative fertilization success of sneaker and paired males. *Sepioteuthis australis* could become a model species for this type of analysis because (1) the multiple mating strategies of males result in females regularly being copulated by more than one male throughout each spawning period; (2) scuba divers can stay close to reproductively active squid without altering their behavior; (3) the regular, short frequency of egg deposition (approximately every 70 s, Fig. 5) ensures that a large amount of data can be collected in a limited time; (4) the translucence of newly deposited egg capsules (Fig. 4f) means that each new capsule can be individually identified; and (5) the low number of eggs in each capsule (~4–5 compared to 100–300 for *Loligo*; Buresch *et al.*, 2001) simplifies the analysis.

Egg deposition and population discrimination

The egg deposition frequencies of *S. australis* from South Australia differed from those reported for other populations of the species and for related species. Deposition of successive egg capsules in a South Australian population of *S. australis* occurred with clear modes at 2.5 s and at 70 s (Fig. 5), whereas a New Zealand population was reported to deposit egg capsules at roughly 5-min intervals (Larcombe and Russell, 1971). Similar, longer intervals between deposition of successive egg capsules have been reported for *S. sepioidea* (2–3 min; Moynihan and Rodaniche, 1982). The very short modal intervals seen here (2.5 s, Fig. 5) are insufficient for “Egg passing” to occur between capsule depositions (requiring ~ 25 s; table 1 in Jantzen and Havenhand, 2003a). Due to the nature of our data (observational) and the egg deposition habitat (seagrass bed), we do not know whether females actually deposited two egg capsules in quick succession or whether, for some reason, they failed to deposit a capsule on the first attempt but did so soon afterwards on a second attempt. Certainly, “Egg passing” behavior did not appear to vary with egg deposition frequency. The causes of variability and plasticity in capsule deposition frequency are not fully understood (but see below); however, it is evident that even at the slower modal frequency seen here (70 s, Fig. 5), *S. australis* females would be capable of depositing well in excess of 50 egg capsules (~ 175 eggs) per hour.

Female body patterns during egg deposition also appeared to differ among different populations of *S. australis*. During egg deposition, females in South Australia folded only the tentacles back (all other arms remained in a “Rigid arms” position; Fig. 4), but females in New Zealand also folded the four lower arms down and back as they approached egg clusters (Larcombe and Russell, 1971). Larcombe and Russell (1971) also saw females pulsing a jet of water towards an egg cluster after egg deposition and interpreted this behavior as aiding in the hardening of the newly deposited capsule. “Peristaltic arm flare” was the only behavior akin to this that we observed. This behavior occurred within a few seconds of egg deposition (Fig. 6), but water was not specifically directed toward the egg clusters. Behaviors similar to “Peristaltic arm flare” have been reported in the cuttlefish *Sepia latimanus* (as “Puffing,” Corner and Moore, 1980) to remove excess “latex-like” substance (= capsule matrix) from among the arms after egg deposition (Corner and Moore, 1980). It is highly likely that “Peristaltic arm flare” in *S. australis* has the same function; the behavior was observed shortly after egg deposition, and white matter was usually expelled from the arms at this time. However, it is also possible that “Peristaltic arm flare” removes spermatophores from the buccal area after fertilization of an egg capsule and before the next mating (providing another avenue for female sexual selection). The material expelled by the female in these “Peristaltic arm flare” behaviors was not analyzed, so we do not know whether it contained sperm/spermatophores or egg capsule matrix.

Investigation of the interval between successive “Peristaltic arm flare” behaviors, between egg deposition and “Peristaltic arm flare,” and between “Egg passing” and egg deposition showed no significant differences among females (ANOVA, $P > 0.05$ in all cases, see Results). The consistency of these behaviors is surprising given the potential for variability, and it indicates that these behaviors may have evolved in response to strong selection pressures.

This study has focused on the interactions of *S. australis* on spawning grounds from South Australia and the implications of the observed behaviors for sexual selection. There is no comparable information on mating behaviors in *S. australis* populations from other regions. We have demonstrated differences in egg deposition characteristics between our data and those from geographically distinct populations in Tasmania and New Zealand. Recently, genetic and behavioral differences between populations of *S. lessoniana* from Japan have caused the classification of this species to be revised (Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994, 1996a, b). Allozyme analysis of *S. australis* has already identified genetic divergence between geographically distinct populations (Triantafillos and Adams, 2001) but stopped short of suggesting that these are subspecies. Further genetic characterization, coupled with detailed behav-

ioral investigations such as those reported here should provide valuable insights, not only into the variability of reproductive behaviors and the potential for differences in sexual selection between populations, but also into the importance of those differences as mechanisms of speciation.

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